

CLAIMS:

1. An isolated DNA sequence other than the structural coding sequence listed in SEQ ID NO:41, SEQ ID NO:43 SEQ ID NO:66 and SEQ ID NO:68, encoding an EPSPS enzyme having the sequence domains:
-R-X₁-H-X₂-E- (SEQ ID NO:37), in which
X₁ is G, S, T, C, Y, N, Q, D or E;
X₂ is S or T; and
-G-D-K-X₃- (SEQ ID NO:38), in which
X₃ is S or T; and
-S-A-Q-X₄-K- (SEQ ID NO:39), in which
X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and
-N-X₅-T-R- (SEQ ID NO:40), in which
X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V.
2. A DNA molecule of Claim 1 in which the K_m forphosphoenolpyruvate is between 2 and 25 μ M.
3. A DNA molecule of Claim 1 in which the K_i/K_m ratio is between 25 and 500.
4. A DNA molecule of Claim 1 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.
5. A DNA molecule of Claim 4 which encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.
6. A DNA molecule of Claim 5 having the sequence of SEQ ID NO:2.

7. A DNA molecule of Claim 5 having the sequence of SEQ IDNO:9.
8. A recombinant, double-stranded DNA molecule comprising in sequence:
- a) a promoter which functions in plant cells to cause the production of an RNA sequence;
 - b) a structural DNA sequence that causes the production of an RNA sequence which encodes a EPSPS enzyme having the sequence domains:
 - R-X₁-H-X₂-E- (SEQ ID NO:37), in which
 - X₁ is G, S, T, C, Y, N, Q, D or E;
 - X₂ is S or T; and
 - G-D-K-X₃- (SEQ ID NO:38), in which
 - X₃ is S or T; and
 - S-A-Q-X₄-K- (SEQ ID NO:39), in which
 - X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and
 - N-X₅-T-R- (SEQ ID NO:40), in which
 - X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V;
- and
- c) a 3' non-translated region which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;
- where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the encoded EPSPS enzyme to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule.

9. A DNA molecule of Claim 8 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.
10. A DNA molecule of Claim 8 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.
11. A DNA molecule of Claim 10 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.
12. A DNA molecule of Claim 9 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.
13. A DNA molecule of Claim 12 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.
14. A DNA molecule of Claim 12 in which the EPSPS sequence is SEQ ID NO:3.
15. A DNA molecule of Claim 14 in which the promoter is a plant DNA virus promoter.
16. A DNA molecule of Claim 15 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

17. A DNA molecule of Claim 14 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.

18. A DNA molecule of Claim 17 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

19. A method of producing genetically transformed plants which are tolerant toward glyphosate herbicide, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant, double-stranded DNA molecule comprising:

i) a promoter which functions in plant cells to cause the production of an RNA sequence,

ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R-X₁-H-X₂-E- (SEQ ID NO:37), in which

X₁ is G, S, T, C, Y, N, Q, D or E;

X₂ is S or T; and

-G-D-K-X₃- (SEQ ID NO:38), in which

X₃ is S or T; and

-S-A-Q-X₄-K- (SEQ ID NO:39), in which

X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X₅-T-R- (SEQ ID NO:40), in which

X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V;

and

- iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;
- where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the polypeptide to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule;
- b) obtaining a transformed plant cell; and
- c) regenerating from the transformed plant cell a genetically transformed plant which has increased tolerance to glyphosate herbicide.
20. A method of Claim 17 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.
21. A method of Claim 20 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.
22. A method of Claim 19 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.
23. A method of Claim 22 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.
24. A method of Claim 23 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

25. A method of Claim 23 in which the EPSPS enzyme is that set forth in SEQ ID NO:3.
26. A method of Claim 25 in which the promoter is from a plant DNA virus.
27. A method of Claim 26 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.
28. A glyphosate-tolerant plant cell comprising a DNA molecule of Claims 9, 12 or 14.
29. A glyphosate-tolerant plant cell of Claim 28 in which the promoter is a plant DNA virus promoter.
30. A glyphosate-tolerant plant cell of Claim 29 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.
31. A glyphosate-tolerant plant cell of Claim 28 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.
32. A glyphosate-tolerant plant comprising plant cells of Claim 31.
33. A glyphosate-tolerant plant of Claim 32 in which the promoter is from a DNA plant virus promoter.

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34. A glyphosate-tolerant plant of Claim 33 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

35. A glyphosate-tolerant plant of Claim 34 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

36. A method for selectively controlling weeds in a field containing a crop having planted crop seeds or plants comprising the steps of:

a) planting the crop seeds or plants which are glyphosate-tolerant as a result of a recombinant double-stranded DNA molecule being inserted into the crop seed or plant, the DNA molecule having:

- i) a promoter which functions in plant cells to cause the production of an RNA sequence,
- ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R-X₁-H-X₂-E- (SEQ ID NO:37), in which

X₁ is G, S, T, C, Y, N, Q, D or E;

X₂ is S or T; and

-G-D-K-X₃- (SEQ ID NO:38), in which

X₃ is S or T; and

-S-A-Q-X₄-K- (SEQ ID NO:39), in which

X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X₅-T-R- (SEQ ID NO:40), in which

X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V;

and

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iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the EPSPS enzyme to enhance the glyphosate tolerance of the crop plant transformed with the DNA molecule; and

b) applying to the crop and weeds in the field a sufficient amount of glyphosate herbicide to control the weeds without significantly affecting the crop.

37. A method of Claim 36 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

38. A method of Claim 37 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

39. A method of Claim 36 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

40. A method of Claim 39 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

41. A method of Claim 40 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

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42. A method of Claim 40 in which the DNA molecule encodes an EPSPS enzyme as set forth in SEQ ID NO:3.
43. A method of Claim 42 in which the DNA molecule further comprises a promoter selected from the group consisting of the CAMV35S and FMV35S promoters.
44. A method of Claim 43 in which the crop plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.
45. A DNA molecule of Claim 9 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17.
46. A DNA molecule of Claim 45 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.
47. A DNA molecule of Claim 9 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.
48. A DNA molecule of Claim 47 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14.

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49. A DNA molecule of Claim 45 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.
50. A DNA molecule of Claim 46 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.
51. A DNA molecule of Claim 47 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.
52. A DNA molecule of Claim 48 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.
53. A DNA molecule of Claim 49 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.
54. A DNA molecule of Claim 50 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.
55. A DNA molecule of Claim 51 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.
56. A DNA molecule of Claim 52 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

57. A DNA molecule of Claim 53 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SSEQ ID NO:44.

58. A DNA molecule of Claim 54 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

59. A DNA molecule of Claim 55 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

60. A DNA molecule of Claim 56 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

61. A DNA molecule of Claim 57 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

62. A DNA molecule of Claim 58 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

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63. A DNA molecule of Claim 59 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

64. A DNA molecule of Claim 60 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

65. A DNA molecule of Claim 53 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

66. A DNA molecule of Claim 54 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

67. A DNA molecule of Claim 55 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

68. A DNA molecule of Claim 56 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

69. A DNA molecule of Claim 65 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

70. A DNA molecule of Claim 66 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

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71. A DNA molecule of Claim 67 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

72. A DNA molecule of Claim 68 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

73. A glyphosate-tolerant plant cell of Claim 29 in which:

(a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;

(b) the structural DNA sequence encodes:

(i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and

(ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and

(c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

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75. A glyphosate-tolerant plant cell of Claim 73 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

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76. A glyphosate-tolerant plant cell of Claim 73 in which the structural DNA sequence comprises:

- (a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and
- (b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

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77. A glyphosate-tolerant plant cell of Claim 74 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

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78. A glyphosate-tolerant plant cell of Claim 75 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

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79. A glyphosate-tolerant plant comprising a DNA molecule of Claims 9, 12 or 14 in which:

- (a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;
- (b) the structural DNA sequence encodes:
 - (i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and
 - (ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and
- (c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

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80. A glyphosate-tolerant plant of Claim 79 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

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81. A glyphosate-tolerant plant of Claim 80 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and

(b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

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82. A glyphosate-tolerant plant of Claim 81 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

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83. A glyphosate-tolerant plant of Claim 82 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

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84.

A seed of a glyphosate-tolerant plant of Claim 32.

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A seed of a glyphosate-tolerant plant of Claim 35.

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86. A seed of a glyphosate- tolerant plant of Claim 79.

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87. A seed of a glyphosate- tolerant plant of Claim 80.

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88. A seed of a glyphosate- tolerant plant of Claim 81.

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89. A seed of a glyphosate- tolerant plant of Claim 82.

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90. A seed of a glyphosate- tolerant plant of Claim 83.

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91. A transgenic soybean plant which contains a heterologous gene which encodes an EPSPS enzyme having a K_m for phosphoenolpyruvate (PEP) between 1 and 150 μM and a $K_i(\text{glyphosate})/K_m(\text{PEP})$ ratio between about 2 and 500, said plant exhibiting tolerance to N-phosphonomethylglycine herbicide at a rate of 1 lb/acre without significant yield reduction due to herbicide application.

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92. Seed of a soybean plant of Claim 91.

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93. In a method for the transformation and regeneration of transgenic plants, the improvement which comprises the use of a glyphosate-resistance marker gene comprising::

- i) a promoter which functions in plant cells to cause the production of an RNA sequence,
- ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

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-R-X₁-H-X₂-E- (SEQ ID NO:37), in which

X₁ is G, S, T, C, Y, N, Q, D or E;

X₂ is S or T; and

-G-D-K-X₃- (SEQ ID NO:38), in which

X₃ is S or T; and

-S-A-Q-X₄-K- (SEQ ID NO:39), in which

X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X₅-T-R- (SEQ ID NO:40), in which

X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V;

and

- iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the polypeptide to render a plant cell transformed with the DNA molecule tolerance to a toxic level of glyphosate.

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94. A method of Claim 93 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

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95. A method of Claim 94 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

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96. A method of Claim 93 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

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97. A method of Claim 96 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

98. A method of Claim 97 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

99. A method of Claim 93 in which the EPSPS enzyme is that set forth in SEQ ID NO:3.

100. A method of Claim 99 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

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